Contribution to micro- and nanoplastics hazard assessment: the lessons learnt from nanomaterials toxicity assessment

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a coordinated approach to assess human health risks of micro- and nanoplastics in food

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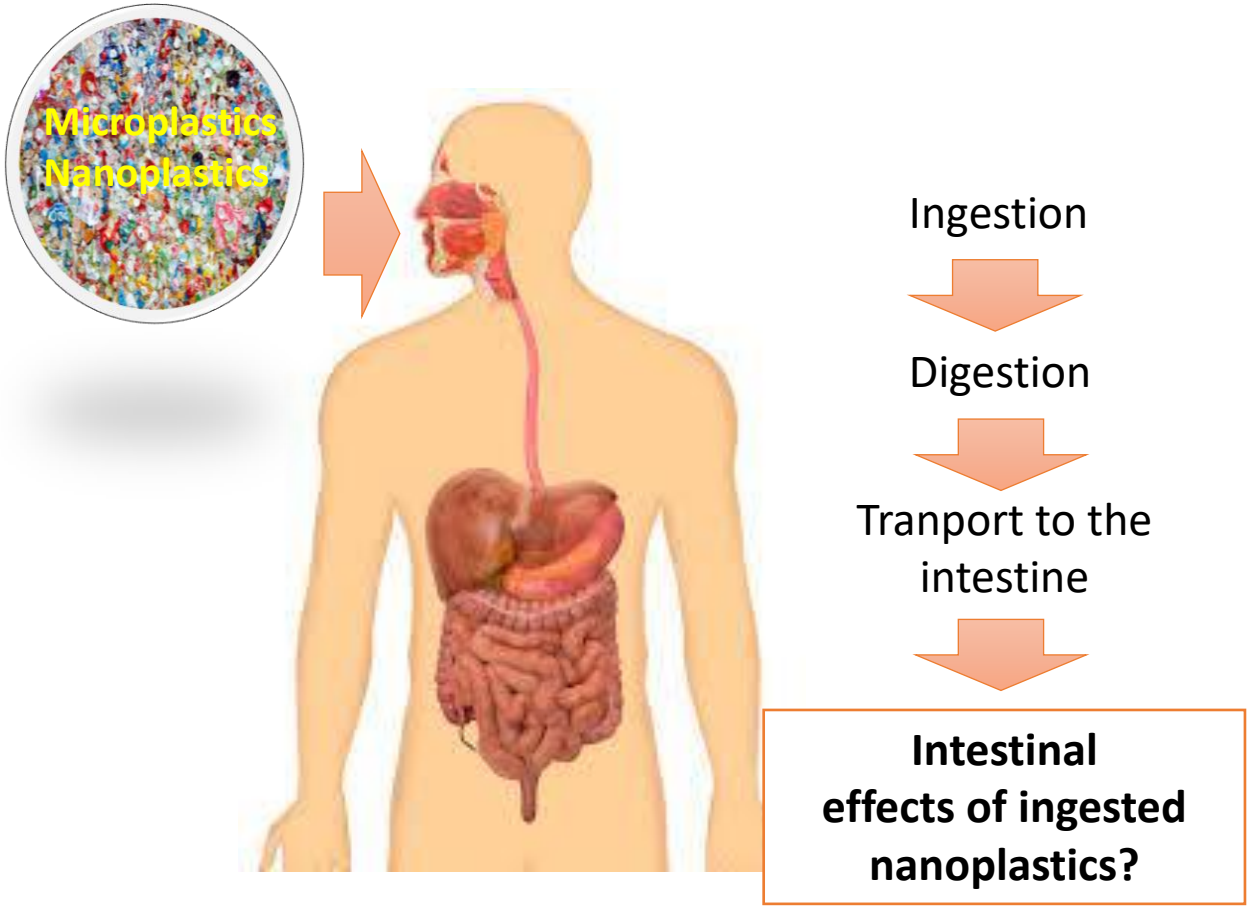
Background

- Micro- and nanoplastics have the potential to accumulate in edible tissues of the exposed organisms (e.g fish), thus entering the human food chain.
- Apart from this indirect exposure route, humans are additionally exposed to tiny plastic particles through water consumption and consumer products that incorporate the so-called microbeads.
- A major question is whether micro- and nanoplastics will have a negative impact on human health. This negative impact may derive from the physicochemical nature of the micro- and nanoplastics, that resemble those of engineered nanomaterials, including their capacity to cross barriers and reach all organs and tissues, inducing deleterious effects e.g., inflammation or genotoxic effects that may lead long-term disease, such as cancer.
- Moreover, these plastic particles might be associated with a wide range of substances, including chemicals present in the plastic composition, e.g., metals, polychlorinated biphenyls and plasticizers or chemicals adsorbed to their surface, which may also be hazardous to human health.

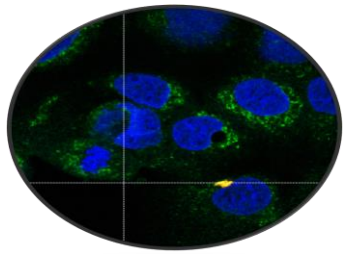


Objective

To propose the use of the testing strategy that has been recently applied to the genotoxicity characterization of nanomaterials in human cell models, e.g., to the hazard assessment of ingested micro- and nanoplastics

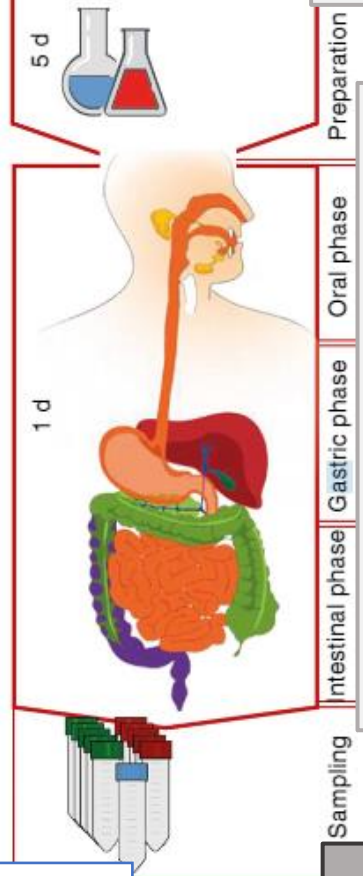
Experimental strategy proposed to assess the adverse effects of micro-nanoplastics to human health (I)

Characterization of primary physicochemical properties
(e.g., size, surface area, chemistry,…)



Uptake by cells

NANOPLASTICS
(Pristine, adorbed with chemicals, metals,…)



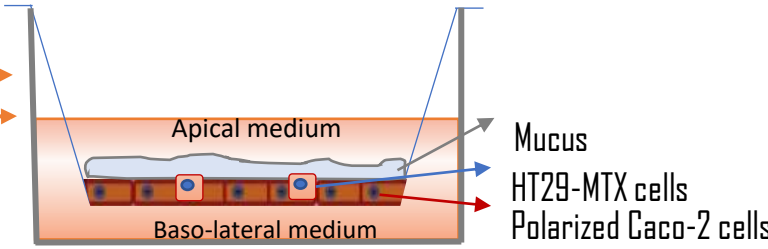
***In vitro* human digestion**
(Brodkorb et al., 2019)

Oral Phase (pH 7)
2 min, 37ºC
Gastric Phase (pH 3)
2 h, 37ºC
Intestinal Phase (pH 7)
2 h, 37ºC

DIGESTED NANOPLASTIC

Characterization of secondary physicochemical properties
(e.g., TEM/EDS and DLS analysis)

Intestinal cellular model:
Polarized Caco-2/HT29 -MTX-E12 Co-cultures



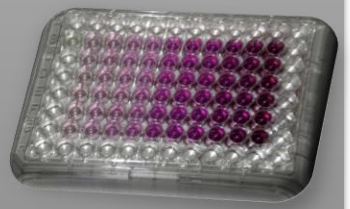
Biological Assays

Experimental strategy proposed to assess the adverse effects of micro-nanoplastics to human health (II)

Biological Assays

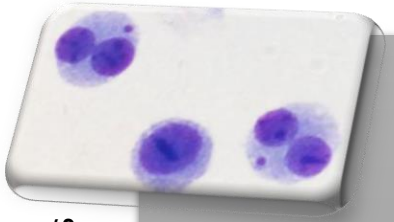
Cytotoxicity

- **Clonogenic Assay**
- **MTT, neutral red uptake assays**



cytotoxicity

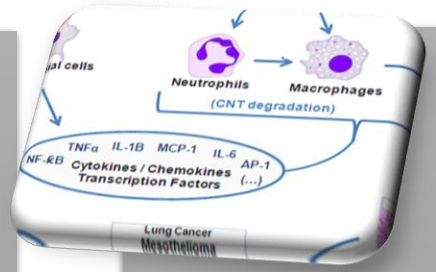
Genotoxicity



Chromosome alterations
DNA damage

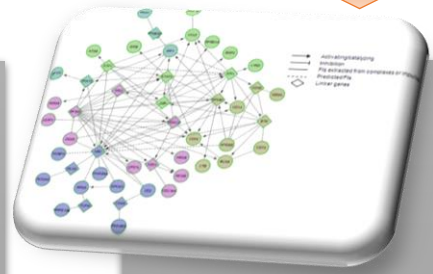
- **In vitro micronucleus assay** (OECD 2016. TG 487)
- **Comet Assay** (Tice et al. 200

Immunotoxicity



- **Cytokines release**
- **Inflammasome activation**

Omics-based approaches



- **Transcriptomics** (Ventura et al., 2018)
- **Epigenotoxicity (e.g., micro RNA profiling)** (Ventura et., 2020)

Data analysis and integration

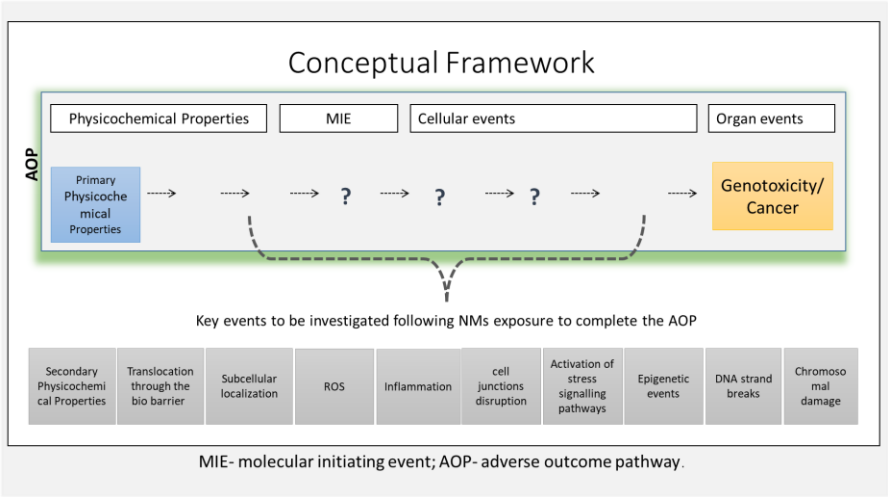
A mechanistic approach towards Adverse Outcome Pathway (AOP) definition

Ingested micro- and nanoplastics

Direct/Indirect human exposure

INTESTINAL TOXICITY

Bowel disease



MIE- molecular initiating event; AOP- adverse outcome pathway.

PREDICTIVE TOXICOLOGY based on New Approach Methods for micro-nanoplastics' hazard assessment in the framework of RA

In conclusion, the use of a predictive toxicology approach based on NAMs will help defining the key events at cellular and molecular levels, in order to characterize the hazard of micro- and nanoplastics while reducing the *in vivo* experimentation, towards their risk assessment